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Preliminary Report

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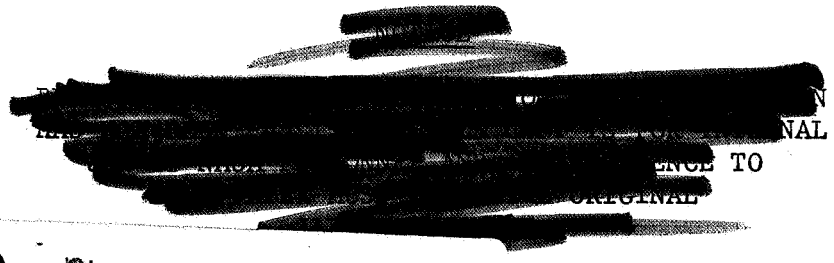

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ABSTRACT

A study of the effect of head-to-foot accelerations of up to 10 g on rabbits is reported. The changes in the electrocardiogram, electroencephalogram, electroretinogram, fractionation of serum protein, eyeground and histology of the brain and the internal organs during accelerations lasting from 20-30 min are observed. On the basis of an analysis of these data, ischemic conditions, presumably of central origin, in certain organs and congestion of blood in the abdominal organs are demonstrated.

1. Introduction

(Motohayashi and Ando)

Investigation of the effect of high gravity on a living organism was /55* begun in this laboratory two years ago. The results obtained to date are only preliminary, but are reported to invite criticism and suggestions concerning the procedures or any other aspects.

*Numbers given in the margin indicate pagination in original foreign text.

Various fields of specialization are represented: brain, nerves, sense organs, conditioned reflexes, heart, circulatory system, body fluid regulation, bioclimatologic change, metabolism, embryology, experimental psychology, and others. Among the researchers are those qualified in physiology, biochemistry, histopathology, and experimental psychology.

One researcher may be examining the mechanism of cardiac contractions by the use of electrophysiological procedures and another studying the factors influencing embryological development, using histopathological procedures. The present plan is for each researcher to offer two to three proposals to this laboratory and to attempt to solve any problems which arise by application of his specialized knowledge and techniques of his field to the study of these problems (as well as to pursue his own specialized work).

The purpose of this procedure is to permit more accurate and reliable examination of a problem by examination of all its aspects by qualified persons, rather than analysis of individual aspects. For example, when a phenomenon such as change in cardiac contraction, respiratory rate or electrocardiogram pattern is observed, a total physiological analysis can be performed, yielding significant data.

This approach is necessarily one of the basic attitudes of research in our laboratory. Although various difficulties arose, the decision was made to attempt an experimental execution of this procedure. /56

To train the persons involved in the various types of research in the modes of operation and analysis, an introductory point of view became the starting point of our project. This is the first report.

2. Experimental Methods

(Motohayashi and Ando)

Detailed procedures are described in each chapter. The summary is as follows:

The experimental animals were rabbits, weighing 2.5-4.0 kg.

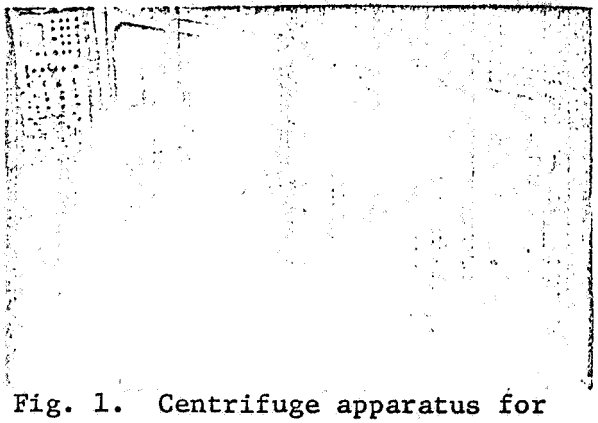


Fig. 1. Centrifuge apparatus for animal experiments

For problems dealing with acceleration loads, we used a centrifugal acceleration apparatus designed in this laboratory. The rotation radius of the centrifuge to the center point where the animal was fixed was 120 cm. The maximum acceleration was 30 g.

For fixation of the animal, the thorax and the four legs of the rabbit were placed inside a metal cylinder

with the neck and thorax in a support lined with spongy,synthetic resin.

Centrifugal acceleration was applied mostly in the head-tail direction; the process is shown in figs. 4 and 5. The size of the load reached 10 g within 4-5 sec., and was maintained according to our requirements for duration of 10 and 20 min in some cases, or until death occurred.

3. Electrocardiology

(Yamada, Okajima, Hori, and Muraki)

(1) Purpose and Procedures. The changes caused by acceleration applied to a living organism, especially the effects on the circulatory system, will be discussed from the point of view of electrocardiography.

Using 2 rabbits, in weight about 3 kg each, needle electrodes were attached to each leg and to a wireless telemeter (model 4321, manufactured by Mitsubishi Denki); recordings were made with an electrometer (model RM-150, manufactured by Nihon Koden). The recordings were made by simultaneously photographing the first and second waves; the recorder speed was 2.5 cm/sec with occasional increases to 5 cm when variations appeared. The head of the rabbit was placed at the centripetal side of the centrifuge and the tail at the centrifugal side. The acceleration load reached 10 g within 4-5 sec after rotation started, and rotation was continued maintaining the 10 g load. Electrocardiograms were taken continuously, starting immediately before rotation, throughout the duration of centrifugation. Rotation was stopped when cardiac contraction ceased, i.e., when death was recognized: 25 min 30 sec in the first case, and 32 min in the second case.

(2) Results. In the first case, as shown in fig. 2, regular sinus pulses were observed with a cardiac contraction rate of 198 min before /57 rotation, and no abnormality in the ST junction was noted.

Two minutes after rotation started, the cardiac contraction rate increased to 297/min, the height of the QRS wave gradually declined, and a lowering of the ST junction was seen for the first time. After 3 min, the cardiac contraction rate returned to 194/min, the rate observed prior to centrifugation, but sinus arrhythmia became marked. The ST junction further decreased, and in some places lowering of ST segments became clearly recognizable.

Furthermore, extrasystoles were observed sporadically. After 10 min, sinus pulsation became regular and the pulse rate increased, 240/min. The height of the P wave increased and sharp peaks were found. After 15 min, although the pulse rate was 207/min and regular, the P waves were negative in both first and second inductions, and the ST segments sagged and were markedly lowered. T waves, therefore, exhibited both a negative and positive nature. After 17 min, the pulse rate was 193/mm and regular, P waves reversed and the T waves became level.

Figure 2

Time (min sec)	Pulse rate (per min)	Pulsation	Wave pattern 1st induction	Wave pattern 2nd induction	Remarks
before rotation	198	sinus pulsation regular			ST normal
1'	297	"			QRS height reduced, ST J decreased
3'	194	sinus arrhythmia			sinus arrhythmia marked, ST J lowered in places, ST segments lowered, Sporadic extrasystole occurred
10'	240	sinus pulsation regular			P wave began to show sharp peaks, sinus arrhythmia disappeared
15'	207	sinus pulsation regular			P wave changed to negative, ST seg lowering marked
17'	193	sinus pulsation regular			T wave became flat
18'	230	sinus arrhythmia			P wave height reduced, ST seg sagged, T wave reversed
18'10"	30-60	sinus pulsation decreased	Same as above		spasms (Adams-Stokes attack)
18'30"	60	auriculer pulsation ceased, ventricular self-movement			

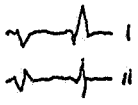
Time (min sec)	Pulse rate (per min)	Pulsation	Wave pattern 1st induction	Wave pattern 2nd induction	Remarks
19'		ventricular self-movement			ventricular groups with Q waves
20'	261	ventricular pulsations frequent			frequent ventricular pulsations began
22'	285	ventricular pulsations frequent			auricular wave-like pattern appeared
23'	125	sinus arrhythmia			ventricular pulsation disappeared; sinus pulsation reappeared; alternate electrical pulses

Fig. 2. Changes in Electrocardiograms of the Rabbit during Centrifugation

Two minutes after rotation started, the cardiac contraction rate increased to 297/min, the height of the QRS wave gradually declined, and a lowering of the ST junction was seen for the first time. After 3 min, the cardiac contraction rate returned to 194/min, the rate observed prior to centrifugation, but sinus arrhythmia became marked. The ST junction further decreased, and in some places a lowering of ST segments became clearly recognizable.

After 18 min, the pulse rate was 230/min and resembled sinus arrhythmia, P wave height was reduced, ST segments became lowered, and T waves became /58 almost negative. After 18 min 10 sec, spasms occurred suddenly (assumed to be Adams-Stokes attacks) and sinus pulsation was as slow as 30-60/min, resembling sinus arrhythmia. However, wave patterns of the QRS group were the same as those observed at 18 min. After 18 min 30 sec, auricular waves disappeared and ventricular self-movement occurred; simultaneously, ST increased in the first induction, and the T wave became level in the second induction. After 19 min, ventricular group waves began to accompany comparatively wide Q waves; in the first induction ST climbed, in the second induction ST sagged. At 20 min, the pulse rate was 261/min of fast ventricular pulses. The ventricular group exhibited a wave pattern that seemed to be continuous from QS waves and moved to the positive T wave. At 22 min, a node was made in the positive T wave, which changed into a wave pattern that resembled auricular waves. At 23 min, the fast ventricular pulses disappeared, and the sinus pulse resumed, but the pulse rate was 125/min showing sinus arrhythmia and in part an alternate electrical pulse. At 25 min 30 sec death of the animal was recognized on the electrocardiogram, and the experiment was terminated by cutting off the electric source of the centrifuge.

In the second case, as shown in figure 3, sinus arrhythmia was already marked even before rotation. The pulse rate was 92/min, the PQ time stretch was 0.08 sec and no deviation was found in ST. After 2 min, the pulse /59 rate was as high as 143/min, and the P wave was found to change to sharp peaks, both in the first and second inductions. After 5 min, sag of the ST junction was first noticed in the first induction, and at 7 min, spasm continued for about 30 sec, clearly indicated on the electromyogram, and no clear ventricular group was found. It was assumed that ventricular contraction had temporarily stopped.

At 8 min, no auricular waves were found, the pulse rate became as low as 30-60 min, and it was considered to be ventricular self-movement. ST segments began to sag slightly. At 8 min 30 sec, sinus pulsations resumed with a pulse rate of 95/min, and it seemed as though conditions had returned to those observed before centrifugation. However, at 10 min, the pulse rate was 65-100/min, typical of arrhythmia, and again no auricular waves were found, but ventricular self-movement was observed. QRS time was stretched to 0.10 sec and there was an unusual pulse pattern. At 10 min 30 sec, the regular sinus pulsation resumed, but a slight extention (0.12 sec) was found in PQ. At 12 min, the T wave began to show increased height, and at 13 min, the T wave climbed further, and from the lower foot of R, a high take-off of the ST-T transient point was found in the first induction. At 15 min, a high take-off at the lower foot of the R and ST segments increased even more, and the PQ time width was extended to 0.15 sec. At 17 min, the ST junction and ST segment exhibited markedly high take-offs showing a pattern like a single-phase wave pattern; the PQ time was further extended to 0.20 sec.

At 20 min, the ST-T moved from the summit of the R wave and became a perfect single-phase wave. At 22 min, second degree atrioventricular block occurred and the pulse rate decreased to 38/min. At 24 min, the base line

Figure 3

Time passed (min sec)	Pulse rate (per min)	Pulsation	Wave pattern 1st induction	Wave pattern 2nd induction	Remarks
before rotation	92	sinus arrhythmia (marked)			No deviation in ST, PQ:0.08 sec
2'	143	sinus arrhythmia			P wave changed into sharp peaks
5'	147	sinus arrhythmia			ST junction lowered
7'		ventricular pulse was assumed to have ceased			spasm continued for about 30 sec, evident on the electromyogram
8'	30-60	auricular pulsation ceased, ventricular self-movement			ST slightly lowered
8'30"	95	regular sinus pulsation			sinus pulsation resumed
10'	65-100	regular sinus pulsation			QRS intervals: 0.10 sec, unusual pulsation
10'30"	95	regular sinus pulsation			sinus pulsation resumed, PQ: 0.12 sec
12'	75	regular sinus pulsation			increased height of T wave
13'	82	regular sinus pulsation			lower foot of R and transitional part of ST and T climbed (high take-off)

Time passed (min sec)	Pulse rate (per min)	Pulsation	Wave pattern 1st induction	Wave pattern 2nd induction	Remarks
15'	81	regular sinus pulsation			high take-off of lower foot of R (ST segment climbed) PQ: 0.15 sec
17'	88	regular sinus pulsation			ST junction and ST segment climbed markedly, single-phase wave PQ: 0.20 sec
20'	50	sinus arrhythmia			single-phase wave
22'	38	sinus beating, 2nd degree atrio ventricular block	Same as above		
24'	60-90				Base line trembled, P wave unrecognizable, QRS group extremely small

Fig. 3. Changes in Electrocardiograms of the Rabbit during Centrifugation

trembled, P waves were unrecognizable, and the QRS group was extremely small, showing arrhythmia of 60-90/min. At 30 min, the base line trembled markedly, with 180/min periodic tremors. The condition became such that it was impossible to tell whether this tremor was ventricular fibrillation or simply instability of the base line. The electric source of the centrifuge was turned off at this point, but at 32 min a ventricular self-movement of 30-40/min was still observed; however, the width of the QRS deflections was extremely small, and the experiment was terminated.

Examining these two examples, we noted that sag of the ST junction in the first and second case had begun at 1 min and 5 min, respectively, and a sag of ST segments began at 3 min and 8 min, respectively (onset of centrifugation). Sharpening of the P wave peaks occurred at 10 min and 2 min, respectively, after beginning of rotation. The spasms in the first case were Adams-Stokes attacks, occurring at 18 min 10 sec; in the second case the spasms lasted for 30 sec at 7 min after beginning of rotation. Sinus arrhythmia occurred occasionally, and auricular cessation and ventricular self-movement occurred at 18 min 30 sec in the first case and at 8 min and at 10 min in the second case.

At the end of the first experiment wide deflections of the Q wave appeared and sag of ST was observed; in the second case, the high take-off of the ST junction and segment gradually increased and finally became a single-phase wave.

(3) Discussion. The ST sag in the first experiment was due to a disorder believed to be caused by a lack of oxygen in the myocardium, and the high ST take-off in the second experiment also suggests a serious myocardial disorder. Furthermore, slow and fast pulses appeared from time to time in both experiments and various abnormal pulsations were found. These can be interpreted as centrally influenced arrhythmias caused by a stimulation due to lack of oxygen in the brain (ref. 1). If this is true, this lack of oxygen in the myocardium and brain may have occurred because of an increase in the blood viscosity in the lower part of the body and lack of blood circulation in the upper part of the body, expecially in the head, due to the physical effect of centrifugal force.

(4) Conclusion. When 10 g of centrifugal force in the head-tail direction was imposed upon two experimental rabbits weighing 3 kg, fast and slow pulses and various other abnormal pulsations were recorded on the electrocardiogram; the development of serious myocardial disorders was shown by the ST deviation and other signs. At the same time, lack of oxygen supply in both cerebral and coronary circulation occurred.

As a result of these conditions, ST sag occurred at 25 min 30 sec in the first case, and ST high take-off occurred at 32 min in the second case; the animal died in each case.

Ref. 1. Ueda, H. et al.: Circulatory and Respiratory Changes induced by Stimulation of the Thalamic Nuclei. Japanese Heart Journal, vol. 1,1, 1960.

These experiments indicate that centrifugal force has a serious effect on the circulatory system of rabbits, but details of the phenomenon require further studies.

4. Electroencephalography and Electroretinography

(Mitarai and Takagi)

(1) Purpose and Methods. In order to observe the physical changes in /60 the central nervous system caused by centrifugal force, the cerebral waves and retinal electrical potential of rabbits were observed. The following results were obtained, using 5 unanesthetized white rabbits weighing 2.5-2.8 kg. The EEGs were obtained by Ag-AgCl electrodes inserted under anesthesia in the front of the head and in the left and right occipital lobes of the brain several days prior to the experiment, and the ERGs, by corneal lens-type electrodes installed after corneal surface anesthesia immediately prior to the experiment; potentials were recorded by wireless telemeter.

Strobe flashes of less than 1 msec were used as a light stimulation for inducing the ERG. The centrifugal apparatus was regulated so that the force reached 10 g at about 5 sec after the beginning of rotation and rotation ceased some 10 sec after being turned off.

(2) Results and Discussion. (I) EEG. The EEG of rabbits placed in the centrifugal apparatus showed that a narrow, fast wave, which could be called an awakening reaction, was predominant, and when a load of + 10 g was added in the head-tail direction, a slightly higher, slow wave of about 3 cycles was added to this (fig. 4). This gradual wave is often observed in a state of rest and constitutes the base line of rabbit cerebral waves at rest. When a + 10 g force is reached and constant speed is maintained, after 1-2 min an awakening reaction similar to that observed prior to centrifugation is seen again in many cases. This indicates the possibility of adaptation to gravitational load.

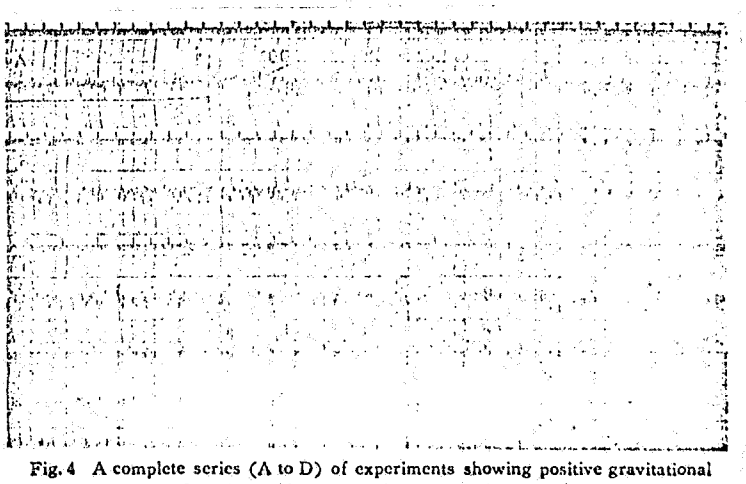


Fig. 4 A complete series (A to D) of experiments showing positive gravitational changes reflected in rabbit's EEG. On and off of centrifuging are indicated by arrows. Calibration; 50 μV, Time; 1 sec.

When the apparatus is turned off and rotation is rapidly stopped, the gradual wave reappeared in the EEG, followed by the appearance of marked spindle group development (fig. 1 D, dotted region), and there was a gradual return to the narrow, fast cerebral wave seen prior to the experiment.

These results differed quantitatively, depending upon the individual differences in the animals, or perhaps upon the conditions of securing the animal in the apparatus, but qualitatively they were found to be the basic changes that normally appear. In other words, the narrow, fast waves and the slow waves followed by spindle group development simply show the stages of cortical excitement levels, the former being high and the latter low. However, these are the changes also observed in quiet, normal rabbits. This fact apparently indicates that the + 10 g load is not strong enough to interfere with central nervous activities. As shown in figure 4, even though the slow wave become predominant (A), or spindle group development occurs (D), cortical activities are apparently maintained in the normal range, because the fast wave group exists more or less the same as in the unstressed animal.

When a load of about 20 g was added to these rabbits, two out of the five died during the load test, another two died immediately after cessation of rotation during the time of spindle group development, and the remaining animal recovered electroencephalographically after cessation of rotation, but died on the following day. Autopsy of the last animal disclosed a large number of hemorrhages in the abdominal cavity. Figure 5 is the electroencephalogram of this rabbit during the experiment. Up to the + 10 g load similar changes were seen, but as rotation was further increased and the load approached + 20 g, the fast wave component suddenly disappeared, resulting in slow waves only (fig. 5 B) and then in complete flatness (fig. 5 B). This fact indicates that each fast wave component and slow wave component shows a different survival time.

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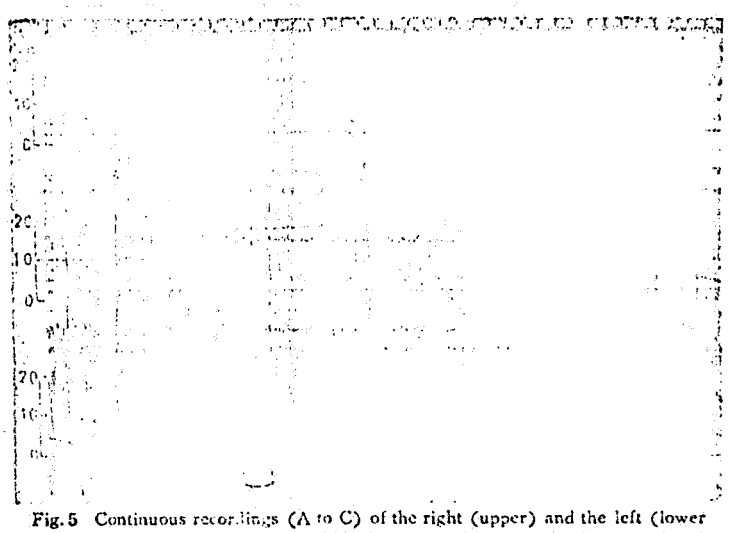


Fig. 5 Continuous recordings (A to C) of the right (upper) and the left (lower trace) occipital EEG in a rabbit showing a characteristic change induced by centrifuge from +10 G to +20 G.

Considering the general point of view that the former participates in cortical activity and the latter in activities of the thalamic relay nucleus group, the difference in survival time may be based on regional differences. When rotation was stopped after the cerebral wave was flattened by the 20 g load, first the slow wave component recovered, then spindle group development occurred, and later the fast wave component gradually appeared and returned to the original state. Since spindle group development is observed in the case of suppression of the ciliary body activation system, evidently very marked suppression occured in this case. We shall confirm this fact later by observing the waves produced by the deep cerebrum.

The changes observed above are very similar to the changes caused by lack of oxygen or low blood sugar. It is obvious that the centrifugal load mainly causes a rapid decrease of cerebral blood flow, and the main reasons for the EEG changes are probably low oxygen and low blood sugar due to this phenomenon. A noteworthy fact is the spindle group development at the time of cessation of rotation; we suggest that the minus gravity may disturb the /62 central mechanism more severely than the positive gravity.

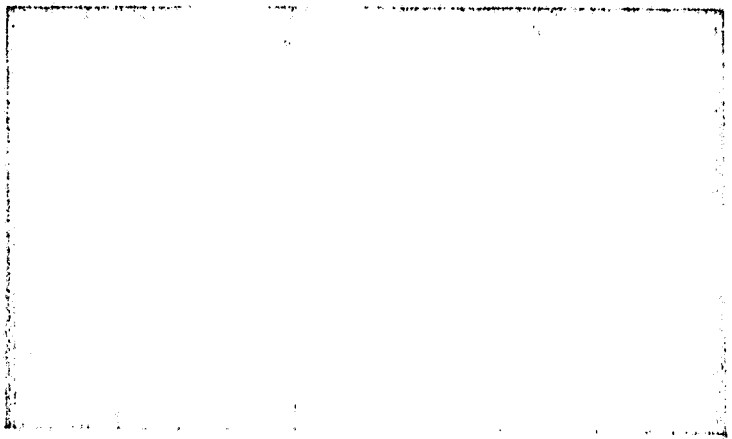


Fig. 6 Effect of centrifuge on the wave shape (upper recording) and the expanded a-wave of the ERG obtained every two minutes before (A), during (B, C, D) and after (E, F) exposure to +10 G.

(II) ERG. Electroretinograms were observed only with the + 10 g load. The results have already been reported (ref. 1). Similar to the cerebral wave which remained within an approximately normal range, there were no marked changes in the ERG. In general, an extension of the a and b waves of the latent period was observed; with respect to the deflection width, the decrease was more marked in the a wave than in the b wave. These are the same changes as those which result from low oxygen content. However, in this experiment the rhythmic small waves frequently disappeared as the centrifugal load was increased; we believe that this is probably a characteristic change. The selective disappearance of rhythmic small waves was found especially in cases of diabetic retinitis (ref. 2), and this has been explained as due to sudden retinal anemia, which is probably the cause of the retinitis. The results of these experiments indicate that a similar mechanism may be operating, and when such phenomena take place severe loss of eyesight probably occurs, as in the latter case.

(3) Subsummary. From these results, we conclude that the changes in EEG and ERG at the time of centrifugal force loading are changes caused by low oxygen content which accompanies sudden anemia. However, rabbits adequately endure the + 10 g load, but die when a + 20 g load is applied, and the cerebral waves change completely to slow and flat waves. Even changes such as these are frequently reversible. Furthermore, a characteristic fact is that spindle group development always occurs at the time of cessation of rotation, and

Ref. 1. Mitarai, G., and S. Takagi: Retinal Potentials in Cases of Low Pressure, Low Oxygen and Centrifugal Force. Journal of Japanese Aerospace Med. and Psy; vol. 2, pp. 90-95, 1964.

Ref. 2. Yonemura, D.: Rhythmic Small Waves Which Appear on ERG. Journal of Japanese Ophthalmology, vol. 66, pp. 1566-1584, 1961.

rabbits die at this stage in many cases. With a + 10 g load, where no marked change was observed in the cerebral waves, the disappearance of rhythmic small waves is often observed on the ERG. We suggest that a blackout at the time of centrifugal loading is due to a deterioration in the retinal mechanisms rather than one in the brain.

5. The Eye

(Suzumura and Miwa)

(1) Purpose and Methods. The anterior of the eye and the eyeground of the test rabbit were viewed by means of ophthalmoscopic and macroscopic examinations before and after centrifugation, and the eyeground was photographed for record. Furthermore, rabbit's eyes were observed by preparing optic discs after the experiment. Changes in tissues were observed by preparing sections after formalin fixation and paraffin embedding, which were then heavily stained with hematoxylin and eosin.

Observations were made 5 min after completion of centrifugation.

(2) Eyeground views. Various parts of the test rabbit's eyeground are compared in figure 7, showing pictures taken before and after centrifugation.

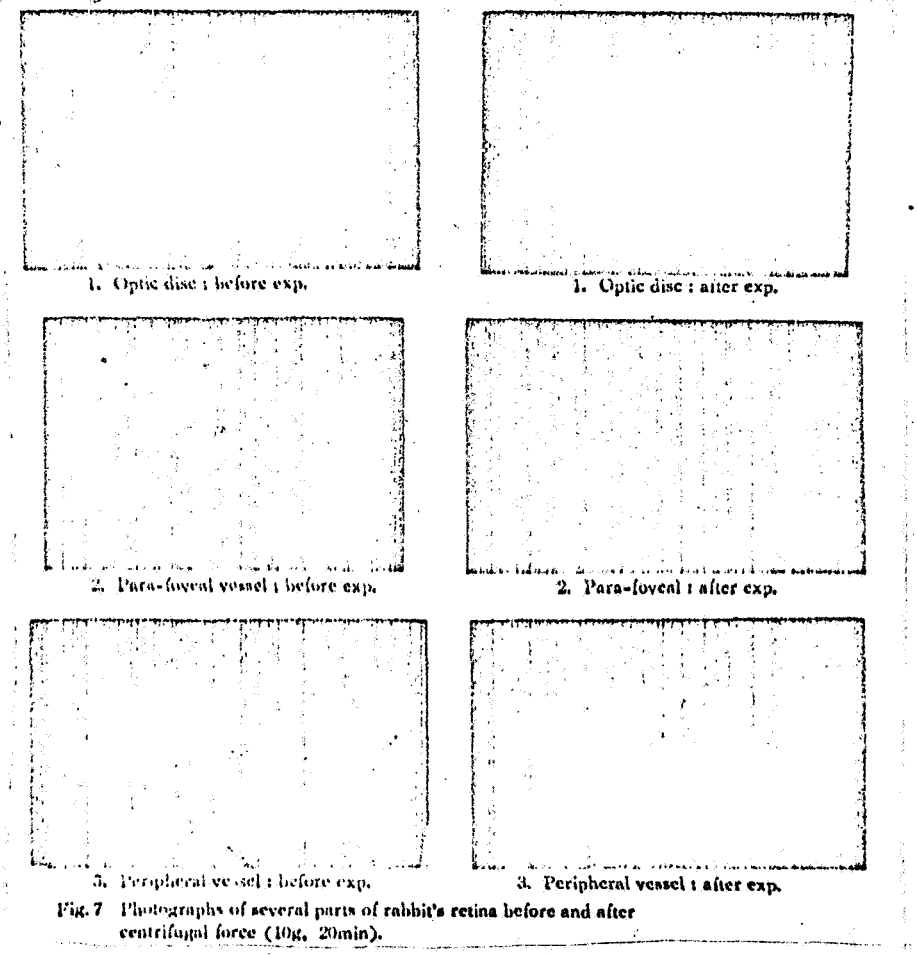


Fig. 7 Photographs of several parts of rabbit's retina before and after centrifugal force (10g, 20min).

Observations near the papillae indicate that after centrifugation the papillae were pale, the blood vessels became unclear, and there was decrease of blood in the vessels. Conditions such as depletion of blood or anemia were also found in other parts, but congestion of blood is recognizable in the choroidal vein found in the diagram near the upper part of the papillae. The farther away from the papillae, the paler the eyeground is after the test. From these observations on the eyeground it appears as if the blood collected at the extreme back of the eyeball around the papillae after centrifugation. However, rather than the total amount of blood that existed prior to the test being congested, it seems that only part of the blood is retained. Observations of the optic fovea indicated no sites of hemorrhage such as broken vessels or thrombi. Therefore, it seems that the flow of blood was dynamically constrained, and the flow from the choroidal vein to outer parts of the eyeball was increased. However, these findings from only two cases are not conclusive. Further observations of the changes which occur during centrifugation are required. It is also not known whether or not the part of the eye where blood collected after centrifugation was influenced by the position of the animal's body after death.

The views of the eyeground after centrifugation are unclear, due mostly to corneal opacity rather than to the influence of centrifugation. The condition of corneal opacity is due mainly to opacity of the corneal epithelium, and it is assumed that this opacity was due in part to poor circulation of the aqueous humor because of the centrifugal acceleration, rather than a simple change after death. However, actual microscopic tests and histological tests of the cornea and chemical tests of the aqueous humor were not done and are planned for future studies.

We shall report on the histological analysis in the future, because /63 there is not sufficient material at the present.

6. Serum Protein Fractionation and Serum Specific Gravity

(Takehara and Suzuki)

As part of an investigation of the changes of body fluid when a centrifugal force was applied to a living organism, the following experiment was performed on rabbits. The accelerating load was 5 g for 10 min. Investigations of the body fluids included serum protein fractionation and serum specific gravity. Blood was collected immediately before and after the centrifugation and 15 min, 30 min, 45 min, and 1 hr thereafter, by cutting the ear lobe six times and collecting 0.5-1.0 ml of blood at a time. Fractionation of protein was done by electrophoresis on cellulose acetate sheets as the support. Specific gravity was determined by the copper sulfate method. The cellulose acetate sheets were Separax (ref. 1), which is reported to be

Ref. 1. Ogawa: Domestic Cellulose Acetate Sheets, Separax. Ekagu no Ayumi (Progress in Medicine), vol. 58, 8 , p. 467, 1964.

valuable for α_1 globulin separation. After electrophoresis at a fixed current the sheets were stained with Poncia 3R and analyzed on an autowriter densitometer.

The experimental results are given in table 1. The percentage of α_1 globulin and β|globulin| in the total protein obtained in six experiments with six rabbits is shown in the table. After thoroughly studying the fluctuations of each fraction, it was decided to give special attention to these two fractions. The α_1 globulin showed a consistent characteristic transition /64 in the low pressure load experiments performed the previous year (ref. 2), and in the present experiment a recognizable pattern of fluctuation was also evident. These α_1 values are shown in figures 8 and 9. When the values obtained immediately before and after centrifugation were compared, an increase was found in four out of six cases, and a slight decrease in two cases, resulting in an increase of the average value.

When the values obtained immediately after centrifugation and 15 min later were compared, a decrease was found in the two cases in which marked increases had been noted during centrifugation and an increase in four other cases, resulting in an increase of the average value. In this comparison the differences in the values of the six cases are large and the fluctuating condition is unstable. In a comparison of the values found 15 min and 30 min after centrifugation, increases were found in three cases and little change in the others. With respect to average values, the percentage of total protein exhibits a perfect straight-line increase up to the 45 min post-centrifugation period. The percentage of total globulin showed a slight decrease between 30 min and 45 min. This is due to a slight increase in globulin in general, but it was not found in all cases. The difference in values decreased between 30 and 45 min, and 45 and 60 min, and the value in each case approaches the average value. The fluctuation also decreased. It is probable that, after emerging from the temporary unstable period caused by the shock, the values are directed toward stabilization, with higher values than at the beginning. The average values showed a sudden /65 increase from 45 to 60 min.

A summary of this course of progress shows that the value increased up to 1 hr after centrifugation, but the course of the increase is not straight and is unstable from 30 to 45 min, later showing a stabilized increase. Our plans are to continue the experiment in order to determine the later course. The β globulin showed high values between 30 and 45 min in two out of six cases, but such an increase was not seen in other cases. In the case of low-pressure load tests the course also passed through an unstable period with

Ref. 2. Takehara, K., and H. Suzuki: Fluctuation of Serum Fractions of Rabbits Caused by Low Pressure--with Special Reference to α_1 Globulin. Kanken Nenpo, vol. 17, p. 99, 1955.

Table 1. Influence of load of gravity upon levels of α_1-, β-globulin fractions and specific gravities of rabbits sera

a) 分画	b) 家兎No. / 採血時間		1	2	3	4	5	6
α_1	d)	前	7.3	3.8	9.3	4.6	2.8	4.9
		後	6.9	6.0	10.4	4.3	3.6	7.6
	後 15 分		10.0	4.7	7.3		5.8	8.9
	〃 30 分		9.6	6.2	9.0	5.0	5.3	8.4
	〃 45 分		8.8	8.4	8.8	6.1	5.3	8.0
	〃 60 分		9.1	10.6	8.4	8.3	7.5	10.2
β	e)	前	11.2	11.6	16.9	9.3	7.8	14.4
		後	7.5	13.7	10.9	12.3	12.7	11.0
	後 15 分		12.4	9.9	18.9		9.4	9.8
	〃 30 分		9.8	14.1	18.8	30.1	7.6	9.7
	〃 45 分		10.3	15.3	15.0	30.7	8.6	10.0
	〃 60 分		10.0	21.1	12.8	26.1	11.2	11.1
c) 比重	f)	前	1025.0	1028.5	1024.5	1024.0	1025.5	1025.0
		後	1024.5	1028.5	1024.0	1025.5	1027.0	1026.5
	後 15 分		1024.5	1028.5	1024.5		1026.0	1025.5
	〃 30 分		1024.5	1028.5	1024.5	1026.0	1026.0	1025.5
	〃 45 分		1024.5	1028.5	1024.5	1026.5	1025.5	1026.0
	〃 60 分		1024.5	1028.5	1024.0	1025.0	1025.5	1026.0

Load of gravity: 5 g, 10 min. Levels of globulin fractions: represented as percentages in total proteins. Specific gravities: determind by copper sulfate solution method.

Legend:

a) Fractions

b) Rabbit No.

Time of Blood Collection

c) specific gravity

d) immediately before centrifugation
immediately after centrifugation
15 min later
30 min later
45 min later
60 min later

e) immediately before centrifugation
immediately after centrifugation
15 min later
30 min later
45 min later
60 min later

f) immediately before centrifugation
immediately after centrifugation
15 min later
30 min later
45 min later
60 min later

later stable fluctuations, but the pattern of the general course was considerably different from that of the acceleration load test. In the present

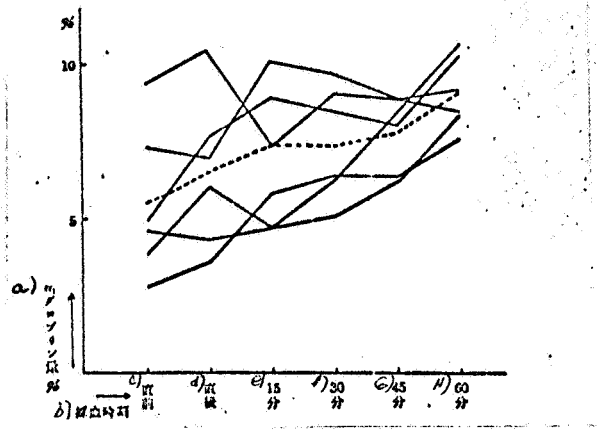
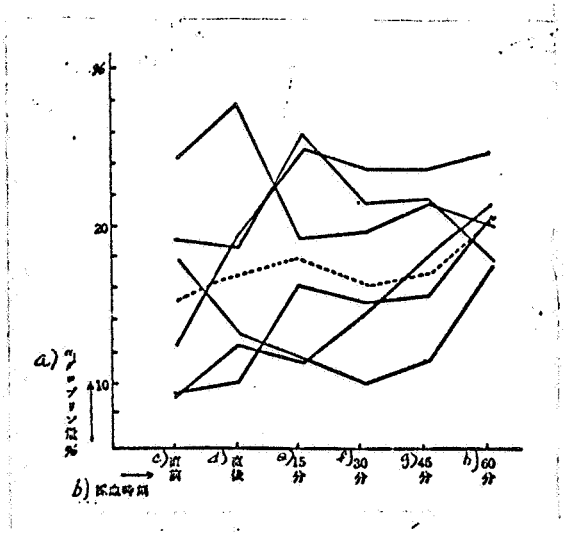


Figure 8. Influence of load of gravity upon levels of α_1-globulin fraction of rabbits sera. Load of gravity: 5 g, 10 min. Levels of α_1-globulin: represented as percentages in total proteins.

Legend: a) α_1 globulin content %
b) time of blood collection
c) immediately before centrifugation
d) immediately after centrifugation
e) 15 min later
f) 30 min later
g) 45 min later
h) 60 min later

experiment no case of death occurred, although there was a case of ataxia after centrifugation.



Legend:

a) β globulin content %
b) time of blood collection
c) immediately before centrifugation
d) immediately after centrifugation
e) 15 min later
f) 30 min later
g) 45 min later
h) 60 min later

Figure 9. Influence of load of gravity upon levels of β-globulin fraction of rabbits sera. Load of gravity: 5 g, 10 min. Levels of β-globulin: represented as percentages in total proteins.

7. Pathological Observations of the Brain

(Murakami and Kameyama)

(1) Experiment 1 (12/14/64) (I), Macroscopic Observations: (microscopic observation of external shape and sectioned surface of fresh specimens).

No hemorrhage was found in either the dura or pia mater and no mechanical injury suggestive of exterior force was found on the surface of the brain. The brain was generally pale and the cerebrum was slightly edematous, but each part of the brain was in its proper proportion and no abnormal pressure marks or topical swellings were found. The frontal, central and occipital cerebral arteries and basilar arteries lacked blood, and the superficial vessels of the brain were small, indicating severe depletion of blood in the brain. No congestion of blood was found in the cerebral veins, and the vascular cavities were flat.

The decrease in the blood content was the same as in the case of the arteries but to a lesser degree, and a considerable amount of blood was still found in the basilar artery [sic, vein].

On the sectioned surface (frontal section), the border between the cerebral cortex and medulla was clear and neither softened foci nor hemorrhagic foci were found. The lateral ventricles were symmetric and both their shape and size were almost normal. No dilation or stenosis was found in the third or fourth cerebral ventricle. Blood marks on section surfaces were markedly reduced in the olfactory lobe and the cerebral surface layer and almost nonexistent in the frontal area. However, in the /66
hippocampus, basal nucleus or interbrain, and midbrain the density was almost normal and only by microscopic observation was a slight congestion of blood found in the small parenchymatous vessels. Along the center part of the hippocampal gyrus to its tail several fresh petechiae were found bilaterally. A small lineal hemorrhage was also observed on the outside of the right olivary nucleus of the medulla oblongata.

(II) Histologic Observations: [observation of sections of nervous tissue stained with either H E (hematoxylin and eosin) or gallocyanine]

The structure of the cerebral cortex was normal, without necrotic foci, and there was no roughening due to dropping of nerve cells. Only in the outer and inner pyramidal layers of the cerebral hemispheres were there occasional cells in which the nucleus was highly stained due to a lack of Nissl substance. Among these cells there were a few which had completely lost their Nissl substance. The deterioration of nerve cells was marked in the hippocampal gyrus, and cells without Nissl substance, in which cytoplasm was evenly and lightly stained, were frequently found on the side of the hippocampus tail.

Nerve cells showing similar deterioration as those seen in the cortex were found sparingly in the cerebral basal nucleus and ophthalmus, but deterioration was only slight in all cases. The nerve nuclei of the lower ophthalmus region, midbrain gray matter, pons, and medulla oblongata were normal and there was no clear picture of deterioration.

In the cerebellum, deterioration of the Purkinje cells was marked and there were many cells with swollen bodies which stained lightly and evenly and lacked Nissl substance.

Although the changes in the blood vessels were not obvious in the cerebral cortex and subcortical white matter, small veins in the lateral ventricular wall, brain stem, and cerebellum showed slight congestion, and perivascular hemorrhages were found in the small veins in the subependymal layer of the lateral ventricular bases and the white matter of the hippocampal gyrus. The cerebral arteries at the base of the brain had contracted and the lumens were narrow; some of them showed a space between the intimal and medial membranes, or loose intimal membranes. In the basilar veins an eosin stainable homogeneous, nonstructural substance, possibly a hemolysin, was found attached to the vascular wall, but there was no distinct thrombus formation.

The cerebral edema and deterioration of the nerve cells in the cerebral cortex and brain stem were all only slight, and it was not possible to establish whether these changes were caused by anoxia due to cerebral depletion of blood, or postmortem changes in the animal. The changes found in the arterial walls and venous walls at the base of the brain were not proven to be caused by hemodynamic changes induced by the experimental treatment or artifacts, and further investigation is necessary. Among the cerebral parenchymatous hemorrhages, the relationship of the small hemorrhages at the medulla oblongata to the experimental treatment is not known, but the small perivascular hemorrhages at the hippocampal gyrus and the base of the lateral ventricles can be considered caused by anoxia due to the experimental treatment, because they are symmetric and both positions are predilected places of hemorrhage due to anoxia. The changes in the nerve cells of the hippocampal gyrus and cerebellar Purkinje cells can be considered caused by anoxia due to the experimental treatment, because they indicate ischemic changes and these nerve cells are most sensitive to anoxia.

(III) Summary of the Cerebral Pathology: It is not clear from this experiment whether the morbid changes in the brain or any direct injury were caused by centrifugal acceleration. The histological changes in the brain indicate early morbid stages due to acute anoxia.

(2) Experiment 2 (1/29/65) (I) Macroscopic Observations: (observation by microscope of external shape and sectioned surface of fresh specimens).

No hemorrhage was found in either the dura or pia mater, and no mechanical injury was found on the surface of the brain. The brain generally appeared to be anemic, but no cerebral edema or morphological abnormalities were found in any part of the brain. The frontal, central and occipital

cerebral arteries, the basilar arteries and each superficial branch were slightly constricted, indicating a slight degree of cerebral depletion of blood. However, the venous system in general was almost normal, and the basilar veins appeared dilated when compared with the control case.

On section surfaces (frontal section) the border between the cortex and medulla was clear and neither softened foci nor hemorrhagic foci were found; the shape and size of the lateral ventricles and the third and fourth ventricles were normal.

Blood marks on section surfaces were markedly reduced in the olfactory lobe and the cerebral hemispheres, but in the hippocampus, basal nucleus or interbrain and midbrain they were almost normal. By observation of enlarged pictures slight vascular dilation and fresh petechiae were found bilaterally on the central region of the hippocampal gyrus. Dilation of small vessels was found in the central part of the cerebellum, but there were no hemorrhages.

These findings are almost the same as in the case of experiment 1, but the cerebral hematogenous injuries were mild. The bilateral petechiae in the hippocampal gyrus were identical to those observed in experiment 1 and are considered due to an anoxia.

8. Pathological Observations of Internal Organs

(Murakami and Chiba)

(1) Methods: After completion of various tests, the body was /67 immediately dissected. After careful macroscopic examination, tissues were fixed in 10 percent formalin, and paraffin sections were made as usual. H E stains, elastic fiber stains, collagenous fiber stain, reticular fiber stains, and polysaccharide stains were made and lipids were stained in frozen sections (Kawamura-Yasaki method).

(2) Observations: With reference to the heart, macroscopically, the right ventricle was dilated and the incisura of the apex was slightly raised; the surface was coarse and the morphology was slightly different. The large and small cardiac veins and the right retroventricular veins were clear, and the myocardium was yellowish brown. Histologically the myocardial fibers were coarse, the nuclei darkly colored, the stroma widened with accompanying edema and the small vessels were dilated and filled with blood; small hemorrhages were occasionally found. The vascular walls situated below the tunica externa were coarse and there were some spaces. The blood components filling the vessels were separated and the blood cells were broken.

Macroscopic observations of the lungs included the following findings. In the first case emphysema was found along the apex of the lung to the front margin of the left lung on the lower margin of the interlobar incisura and on the thorax surface slightly higher than the lower margin of the lower lobe;

the lower lobe was dark red and hard in texture. In the right lung emphysema was found in the upper lobe and the apex; upper, middle, and lower lobes were hard in texture and dark red, and localized emphysema was found on the thorax and mediastinal surfaces of the lower lobe. The capacity was small when compared with the left lung.

In the second case, emphysema was found on the lower margin of the lower lobe along the apex to the front margin up to the thorax surface, in both the left and right lungs, which were thin transparent and edematous. Each pulmonary lobe was hard in texture and severely congested.

In the third case the left lung exhibited emphysema along the apex to the front margin, and although the upper and lower lobes were light red, there were dark red foci, the texture was hard, and localized emphysema was found on the lower margin of the lower lobe. In the right lung, emphysema was found along the apex to the front margin, along the interlobar incisura of the middle lobe, along the immediate lower margin of the interlobar incesura of the lower lobe and localized on the mediastinal surface; the lung was light red in general, but there was a place showing a wedge-like dark red area in the lower lobe. Histologically there were slight differences in the upper, middle and lower lobes.

Summarizing observations of these three cases gives us the following picture. The mucosal cilia of the large bronchi were unclear, the epithelial cells were small, the nuclei were darkly colored and the muscle fibers of the wall were coarse. In one case the mucosal epithelium was detached from the basal membrane. In the relatively peripheral bronchi the mucosa was inflated, or was adenomatous and inverted. The mucosal epithelial cells adjoining the acinous alveolar septum and alveoli were inflated, and the inner cavities of some of them were occuluded. In some of the branched bronchi red blood cells or denuded mucosal epithelium were found in the tubular cavity. As for the blood vessels, the inner cavity of the large vessels had become hollow and narrow; the endothelial cells were dark and vacuoles were found as well as vitrification of muscle fibers and the formation of spaces due to shrinkage. In the inner membranes broken blood cells were agglutinated, or in some cases the blood components in the blood vessels had separated. Some of the relatively peripheral vessels contained large quantities of blood cells, and in some cases the inner membrane had become papillary and incrassated inward.

The pulmonary tissues generally show the following three histological changes. In some place the pulmonary acini at the center were oppressed and the alveoli had become small, accompanying severe congestion of the blood or, because of congestion the alveolar epithelium accumulated in a lump. In some places a localized condition of airless lungs was seen, and the pulmonary tissues were accompanied by progressive hemorrhages. In some places the margins were ischemic with edema, and the alveoli largely dilated or shrunk; the dilated alveoli adhered to one another resembling cysts. The changes consisted of all these individual features variously intermixed.

Examination of the stomach showed macroscopically that the cardia was contracted, and the muscle layer was thick. However, histologically the mucosal epithelial cells were atrophic, the muscle fibers were very fine and the small interstitial vessels were dilated. The spleen showed severe congestion of blood in the head in some cases, but generally it did not show any marked changes in particular. Histologically the structure was coarse, the follicles were atrophic and the spleen pulp was coarse, but the sinus was dilated and numerous blood cells were found. Recticular cells had increased in the blastema center of the follicles in some cases, and there were some cases where small hemorrhages were found in the tunic. The spleen tissues facing the internal organs lacked blood, compared with the spleen tissues facing the back.

The liver did not show any special morbid changes in appearance. Histologically the lobules were clear, but the hepatic cells were atrophic, the cellular funiculi were unclear, and the cells were lumpy in places. The central veins were dilated and many blood cells were seen in some cases. The portal branches were torn and hemorrhages had occurred in some cases. In the kidney no special changes were observed macroscopically, except opacity and congestion of blood. Histologically, however, the glomeruli were large and Henle's loops were clear and filled with numerous blood cells; in one area blood cells were found only in Bowman's capsule. The small ureter had severely deteriorated and vitrious substances had formed in the inner cavity. Macroscopically the adrenal gland in all cases was the size of a soy bean, yellow, with an enlarged cortex, and in some cases with small hemorrhages in the cortex. Histologically the globular cells were atrophic, but the fascicular cells enlarged, and in some cases small hemorrhages were found in the fascicular zone. In the pancreas no abnormalities were found macroscopically, but histologically the zymogen granules occluding the inner cavity of the acini were extremely small and few in number. In some cases small interstitial vessels were expanded and filled with blood cells; small hemorrhages were found in the stroma. In the arteries no marked changes were found macroscopically, but histologically a few broken red blood cells were attached to the membrane, and atrophy or expansion of the intimal cells /68 and formation of spaces in the muscle fibers of the media were observed.

(3) Discussion from the Pathological Point of View of the Internal Organs: Morphologic changes observed in a living organism subjected to sudden acceleration forces consisted of vascular changes caused by abnormal blood flow and its subsequent changes. In other words, when an acceleration force is added to a living organism, the vascular balance of the blood pressure is lost, thus damaging the vascular resilience maintained by the balanced pressure, and the peripheral vessels show depletion of blood. We believe that the blood flow influenced by the centrifugal force runs along the lower side of the blood vessels, thus causing a separation of the blood components due to temporary stopping or delay of the blood flow or blood cells are broken and metabolic abnormalities occur, causing the adhesion of the broken cells to the intimal membrane. This observation was proven by studying the large vascular branches in the aorta and pulmonary system.

Although few aggregated blood cells were found in the lumen of the aorta, abnormal blood cells were attached to the intimal membrane, the cells in the intimal membrane were flattened, and vacuolization had occurred. There was cessation or loss of structure and that of the medial and adventitial membranes was disturbed, the muscle fibers being extremely atrophic; small spaces had formed. The vascular walls in the pulmonary system had expanded, and occlusion, stenosis, and expansion of the lumens were found. The occluded or stenotic vessels contained in their lumens separated blood components, and the blood had deviated slightly to one side. The broken red blood cells were attached to the intimal membrane here and there, the intimal membrane cells were flattened or inflated, and below this, vacuolization occurred and fibers were disordered in the medial and adventitial membranes, spaces had formed and polypous incrassations of the intima caused by the loss of pressure balance were found. In the case of large vessels that were dilated and contained no blood at all, their walls were thin, the muscle fibers were atrophic, and small spaces had formed.

The pulmonary system developed circulatory disorders influenced by the vascular changes described above, and various morbid changes were furthered by the disturbances of the internal pulmonary pressure and respiration. The place considered to be more or less in the center of the pulmonary system and the pulmonary acini ajacent to large bronchi or large vessels became small, but the alveoli were clear and in a contracted state accompanying the congestion of the blood; these would at last become airless lungs if these conditions of morbid changes continued. Now these morbid changes were localized, and the alveolar epithelium had accumulated, accompanying the congestion of the blood. In the case of emphysema localized at the pulmonary apex, front margin and lower margin, the alveoli were abnormally expanded and were depleted of blood, or the alveoli were depleted of blood because of its contraction and were edematous. This is due to a circulatory disorder, but in the case of expanded alveoli adhering to each other and changing into cyst-like formations we believe some respiratory disorder may have had a slight influence.

As far as the morbid changes of the bronchi are concerned, the mucosa was peeled from the basal membrane and the vascular walls became very thin where the branches were relatively large. Approaching the peripheral region, the mucosal epithelial cells were expanded. At the transition point between the bronchi and alveoli, the inner cavities were occluded in places. This proves that the bronchi are also influenced by the addition of accelerating forces. Blood cells were found localized in the bronchi; they probably entered the bronchi when the alveolar capillary vessels broke. This can be established by the fact that in the third case hemorrhage occurred in the oral cavity and blood was found on the abdominal coat. As far as the organs are concerned, the tunic became thin in all cases, and a little space appeared between the tunic and tissue--this phenomenon is probably the effect of the accelerating force. A similar interpretation can be made where small vessels in the tunic expanded or tore and little hemorrhages were found. The fact that the peripheral vessels in the organs showed depletion of blood was proven histologically, and this observation was verified by the results in the spleen, liver and kidney.

9. Conclusions

The most difficult thing in an attempt of this kind of work was the adjustment of time for each researcher. They have their own problems to pursue and had difficulty in fitting in the time for this work. However, when the direction of the investigation and its specific methods are studied in advance and day, time and duties are scheduled properly, the work can be performed quite smoothly.

When the purpose and methods of the research are discussed and the results are studied, each researcher has an opportunity to listen to the opinions of specialists in various fields, thus enlarging his insight and increasing his intimate relationship as a man. Aside from the academic achievement, it seems that many advantages exist in this approach. All members are trying to develop this approach by planning more of this type of experiment for the future.

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